

CLAIMS

1. A variant of a parent fungal cutinase, which variant:

- a) comprises substitution of at least one amino acid residue corresponding to position A4, T29, A88, N91, A130, Q139, I169, I178 or R189 in the cutinase of *Humicola insolens* strain DSM 1800 (*H. insolens* cutinase numbering), and
- b) is more thermostable than the parent cutinase.

2. The variant of the preceding claim which comprises the substitution A4V, T29M/I/C, A88H/L/V, N91H, A130V, Q139R, I169A/G/T/V, I178V or R189A/H/V.

3. A variant of a parent fungal cutinase, which variant:

- a) comprises substitution of at least one amino acid residue corresponding to Q1C/L, L2K/Q/V, G8D, S11T, N15D, A16T, V38H, S48E/K, H49Y, L66I, S116K, S119P, G120D, T164S, T166M/I or L167P in the cutinase of *Humicola insolens* strain DSM 1800 (*H. insolens* cutinase numbering), and
- b) is more thermostable than the parent cutinase.

4. The variant of any preceding claim wherein the parent cutinase:

- a) is native to a filamentous fungus, particularly a strain of *Humicola* or *Fusarium*, particularly *H. insolens* or *F. solani pisi*, most particularly *H. insolens* strain DSM 1800,
- b) has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800, or
- c) has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800, particularly at least 70 % homologous, more particularly at least 80 % homologous.

5. A variant of the cutinase of *Humicola insolens* strain DSM 1800, which variant:

- a) comprises a substitution of amino acid residue N44, I168 or L174, and

b) is more thermostable than the parent cutinase.

6. The variant of claim 5, which comprises the substitution N44D, I168F or L174F.

7. The variant of any preceding claim which has one to twenty of such substitutions, particularly 2-15.

5 8. The variant of any preceding claim comprising substitutions corresponding to:

a) S48E +A88H +N91H +R189V

b) Q1L +L2K +G8D +N15D

c) N44D +A130V

d) Q1C +L2V +G120D

e) A88L +R189A

f) S48E +L66I +A88L +I169A +R189H

g) A88V +S116K +S119P +Q139R +I169V +R189V

h) A88V +R189A

i) S48K +A88H +I169G +R189H

j) Q1L +L2Q +A4V +S11T

k) T164S

l) L174F

m) H49Y

n) Q1L +L2K +G8D +N15D +S48E +A88H +N91H +R189V

o) Q1L +L2K +G8D +N15D +N44D +A130V

p) Q1L +L2K +G8D +N15D +S48E +A88H +N91H +A130V +R189V

q) G8D +N15D +A16T

r) A130V

s) Q1C +L2V

t) G8D +N15D +A16T

u) G8D +N15D +S48E +A88H +N91H +A130V +R189V

v) G8D +N15D +T29M +S48E +A88H +N91H +A130V +R189V

w) G8D +N15D +T29I +S48E +A88H +N91H +A130V +R189V and/or

- x) G8D +N15D +T29C +S48E +A88H +N91H +A130V +R189V
- y) G8D +N15D +S48E +A88H +N91H +A130V +L174F +I178V +R189V
- z) G8D +N15D +S48E +A88H +N91H +A130V +T166M +I168F +R189V
- aa) G8D +N15D +S48E +A88H +N91H +A130V +T166I +L167P +R189V
- bb) G8D +N15D +V38H +S48E +A88H +N91H +A130V +I169T + R189V
- cc) G8D +N15D +V38H +S48E +A88H +N91H +A130V +R189V
- dd) G8D +N15D +T29M +S48E +A88H +N91H +A130V +T166I +L167P +R189V

9. The variant of any preceding claim which further comprises at least one amino acid substitution at positions corresponding to Q1, L2, E6, E10, S11, A14, N15, F24, L46, E47, R51, D63, L138 and/or E179 (*H. insolens* cutinase numbering).

10. The variant of the preceding claim comprising at least one substitution corresponding to Q1P, L2V, E6Q, E10Q, S11C, A14P, N15T, F24Y, L46I, E47K, R51P, D63N, L138I and/or E179Q (*H. insolens* cutinase numbering).

11. The variant of the preceding claim comprising substitutions corresponding to E6Q +A14P +E47K +R51P +E179Q.

12. The variant of any preceding claim which has hydrolytic activity towards terephthalic acid esters, particularly towards cyclic tri(ethylene terephthalate) and/or Terephthalic acid bis(2-hydroxyethyl)ester dibenzoate (BETEB).

13. The variant of any preceding claim which has a denaturation temperature which is at least 5° higher than the parent cutinase, particularly measured at pH 8.5.

14. A DNA sequence encoding the variant of any preceding claim.

15. A vector comprising the DNA sequence of the preceding claim.

16. A transformed host cell harboring the DNA sequence of claim 14 or the vector of claim 15.

17. A method of producing the variant of any of claims 1-13 comprising

- a) cultivating the cell of claim 16 so as to express and optionally secrete the variant, and
- b) recovering the variant.

18. A method of producing a cutinase variant, which method comprises:

- a) selecting a parent fungal cutinase,
- b) altering at least one amino acid residue in the parent cutinase substitution, deletion or insertion at a position corresponding to A4, G8, A16, T29, V38, N44, S48, H49, L66, A88, N91, S116, S119, G120, A130, Q139, T164, T166, L167, I168, I169, L174, I178 or R189 or in a region comprising such position in the *H. insolens* cutinase (*H. insolens* cutinase numbering) and optionally at other positions to obtain a variant cutinase,
- c) testing the thermostability of the variant cutinase,
- d) optionally repeating steps b-c,
- e) selecting a variant cutinase having higher thermostability than the parent cutinase, and
- f) producing the selected variant cutinase.

19. The method of claim 18 wherein the amino acid alteration is done by localized random mutagenesis in a region comprising at least one of the indicated positions.

20. The method of claim 18 wherein the amino acid alteration is done by point specific mutation in at least one of the indicated positions, particularly by substitution at one, two, three, four, five or six of said positions.

21. The method of any preceding claim wherein the selected variant cutinase has a denaturation temperature which is at least 5° higher than the parent cutinase, particularly measured at pH 8.5.

22. The method of any preceding claim wherein the parent cutinase:

- 5 a) is native to a filamentous fungus, particularly a strain of *Humicola* or *Fusarium*, particularly *H. insolens* or *F. solani pisi*, most particularly *H. insolens* strain DSM 1800,
- b) has an amino acid sequence which can be aligned with the cutinase of *H. insolens* strain DSM 1800, or
- 10 c) has an amino acid sequence which is at least 50 % homologous to the cutinase of *H. insolens* strain DSM 1800, particularly at least 70 % homologous, more particularly at least 80 % homologous.

23. A process for enzymatic hydrolysis of a cyclic oligomer of ethylene terephthalate, which process comprises treating the cyclic oligomer with a variant of a parent fungal cutinase, which variant comprises substitution of one or more amino acid residues at a
15 position corresponding to A4, G8, A16, T29, V38, N44, S48, H49, L66, A88, N91, S116, S119, G120, A130, Q139, T164, T166, L167, I168, I169, L174, I178 or R189 in the *H. insolens* cutinase (*H. insolens* cutinase numbering).

24. The process of the preceding claim, in which the cyclic oligomer is cyclic tri(ethylene
20 terephthalate).

25. The process of claim 23 or 24 wherein the treatment is done above 55°C.

26. The process of any of claims 23-25 wherein the cyclic oligomer is present in and on the fibers of a polyester containing fabric or yarn.

27. The process of any of claims 23-26 which further comprises subsequently rinsing the fabric or yarn, particularly rinsing with an aqueous solution having a pH in the range of from about pH 7 to about pH 11.

28. A process for improving the functional finish of a PET-containing yarn or fabric comprising

- a) treating the yarn or fabric with a variant of a parent fungal cutinase, which variant comprises substitution of one or more amino acid residues at a position corresponding to A4, G8, A16, T29, V38, N44, S48, H49, L66, A88, N91, S116, S119, G120, A130, Q139, T164, T166, L167, I168, I169, L174, I178 or R189 in the *H. insolens* cutinase (*H. insolens* cutinase numbering)., and
- b) subsequently treating the yarn or fabric with a finishing agent selected from the group consisting of softeners, anti-crease resins, anti-static agents, anti-soiling agents.

29. A process for dyeing polyester fabric or yarn, comprising:

- a) treating the fabric or yarn with a cutinase having a thermal denaturation temperature of 65°C or higher at pH 8.5; and
- b) dyeing the treated fabric with a reactive dye or a disperse dye.

30. The process of the preceding claim wherein the cutinase is a variant of a parent fungal cutinase, which variant comprises substitution of one or more amino acid residues at a position corresponding to A4, G8, A16, T29, V38, N44, S48, H49, L66, A88, N91, S116, S119, G120, A130, Q139, T164, T166, L167, I168, I169, L174, I178 or R189 in the *H. insolens* cutinase (*H. insolens* cutinase numbering).

31. A detergent composition comprising a surfactant and the variant of any of claims 1-13.

32. A fungal cutinase having a peptide extension AAVDSNHTPAVPELVAR (SEQ ID NO: 2) at the N-terminal.